High-throughput downstream processing using cellulose fiber chromatography and ÄKTA™ chromatography systems

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Introduction
Developing a process or looking for a clone of interest often requires screening large numbers of samples. Many analyses require purified proteins, and often substantial hands-on time is required for purification. The novel cellulose fiber technology (known as Fibro) may be used with ÄKTA chromatography systems and autosamplers to reduce process time significantly compared with conventional chromatography. Fibro chromatography enables residence times of seconds, with full chromatographic runs in a few minutes per cycle. Here, we describe a versatile solution that significantly increases throughput by completing the purification process in minutes rather than hours.

What is Fibro chromatography?
GE Healthcare Life Sciences Fibro technology also known as Purify technology utilizes the high flow rates and high capacities of cellulose fiber. Fibro technology offers improved protein capture compared to conventional chromatography, with residence times measured in seconds rather than minutes (Fig 1 and Fig 2).

A full chromatographic mAb capture cycle can be completed in a few minutes
A 0.4 mL laboratory-scale Fibro unit can run a full chromatographic cycle in a few minutes. With a simple plug-and-play setup, bidirectional flow, and high multicycle lifetime, the laboratory-scale Fibro unit is a suitable scouting tool for understanding a process and for optimizing conditions—or for generating hundreds of milligrams of material when cycled. Dynamic binding capacity (DBC) of the protein A Fibro unit at different residence times is shown in Figure 4.

Rapid multicycling while maintaining high recovery
Remaining dynamic binding capacity (DBC) for 250 cycles without cleaning in place (CIP) included in each cycle can be performed between each sample to avoid cross-contamination and to ensure no pressure increase.

Manage large numbers of samples with automation
It is simple and quick to run many samples in serial mode when running the Fibro units on an ÄKTA system connected to an autosampler. A method can be set up in the UNICORN™ software and automatically repeated, matching the number of samples loaded in the autosampler.

Significantly reduced process development time compared to conventional chromatography
Process development time can be significantly reduced due to the short cycling time of Fibro chromatography. Compared to conventional chromatography columns, PreDictor™ RoboColumn™ units decrease process development time and material consumption to a large extent. Fibro chromatography further reduces process development time. In contrast to PreDictor RoboColumn units, full chromatograms are provided, giving improved quality monitoring. A comparison of the optimization of the mAb capture step is shown in Table 1.

Table 1. Optimization study of the chromatography purification process comparing the HiTrap™ Maltdectect™ S1 run on ÄKTA avant 25, PreDictor RoboColumn run on Freedom Evo fast 200, and laboratory-scale Fibro units run on ÄKTA avant 25 chromatography system.

<table>
<thead>
<tr>
<th>HiTrap column</th>
<th>PreDictor RoboColumn</th>
<th>Fibro unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples per run</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>Time per run (s)</td>
<td>~10</td>
<td>~10</td>
</tr>
<tr>
<td>Run needed for purifying 12 samples</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Total time (min)</td>
<td>~250</td>
<td>~360</td>
</tr>
<tr>
<td>Protein recovered (mg)</td>
<td>70 ± 5</td>
<td>100 ± 15</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>&gt;90%</td>
<td>&gt;80%</td>
</tr>
</tbody>
</table>


Conclusions
We describe an attractive tool for early-stage development that will ultimately reduce time-to-product and cost.

- Fibro technology delivers a full chromatographic mAb capture cycle in minutes. The first solution that will be available will be a laboratory-scale Fibro unit with immobilized Maltdetect™ PrismA ligand.
- Large sample volumes can be loaded at high flow rate while maintaining high DBC.
- Serial setup with autosampler on ÄKTA systems provides full chromatograms in high-throughput mode, managing large numbers of samples in an automated way.

Fig 1. Conventional chromatography: diffusive flow. Mass transfer is restricted by the diffusivity of molecules through the pores in the beads, and process flow is restricted by the rigidity of the beads.

Fig 2. Fibro chromatography: convective flow. Graded fast mass transfer by convective flow while process flow is only restricted by the size of the pores in the matrix material.

Fig 3. Protein A binding profile on standard cell culture mAb feed of 5 mg/mL purified on a 0.4 mL protein A, laboratory-scale Fibro unit: 1.5 s residence time, 96% recovery, 211 ppm host cell protein (HCP).

Fig 4. Flow rate and pressure handling properties of a 0.4 mL protein A Fibro unit. DBC was determined by bleeding 1.5 mL/hr, purified mAb to 10% breakthrough.

Fig 5. DBC performance of protein A Fibro unit over 250 cycles. Sample clarified cell culture feed with a mAb titer of 5.0 mg/mL, with no cleaning in place (CIP) included in each cycle. 10 mL/hr was used for all phases in a 0.4 mL adsorbent volume, 2 min/cycle, to a 10 mL/hr load challenge.

Fig 6. This picture illustrates a set-up facilitating high-throughput purification using the autosampler AKA-100 Biofill, sample pump (middle top) fraction collector (middle bottom), and ÄKTA pure 25 system (right).

Fig 7. A comparison of the optimization of the mAb capture step is shown in Table 1.

Fig 8. A full chromatographic mAb capture cycle can be completed in a few minutes.